Response of cardiac mast cells to atrial natriuretic peptide

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Murray DB, Gardner JD, Levick SP, Brower GL, Morgan LG, Janicki JS. Response of cardiac mast cells to atrial natriuretic peptide. Am J Physiol Heart Circ Physiol 293: H1216–H1222, 2007. First published April 13, 2007; doi:10.1152/ajpheart.01388.2006.—Previously, our laboratory demonstrated that cardiac mast cell degranulation induces adverse ventricular remodeling in response to chronic volume overload. The purpose of this study was to investigate whether atrial natriuretic peptide (ANP), which is known to be elevated in chronic volume overload, causes cardiac mast cell degranulation. Relative to control, ANP induced significant histamine release from peritoneal mast cells, whereas isolated cardiac mast cells were not responsive. Infusion of ANP (225 pg/ml) into blood-perfused isolated rat hearts produced minimal activation of cardiac mast cells, similar to that seen in the control group. ANP also did not increase matrix metalloproteinase-2 activity, reduce collagen volume fraction, or alter diastolic or systolic cardiac function compared with saline-treated controls. In a subsequent study to evaluate the effects of natriuretic peptide receptor antagonist on volume overload-induced ventricular remodeling, ananiti was administered to rats with an aortocaval fistula. Comparable increases of myocardial MMP-2 activity in treated and untreated rats with an aortocaval fistula were associated with equivalent decreases in ventricular collagen (P < 0.05 vs. sham-operated controls). Cardiac functional parameters and left ventricular hypertrophy were unaffected by ananiti. We conclude that ANP is not a cardiac mast cell secretagogue and is not responsible for the cardiac mast cell-mediated adverse ventricular remodeling in response to volume overload.

atrial natriuretic factor; isolated heart; extracellular matrix; ventricular remodeling; fistula

Previously, we were able to demonstrate a causal effect between resident cardiac mast cell degranulation and induction of matrix metalloproteinase (MMP) activation, which leads to adverse ventricular remodeling in the aortocaval (AV) fistula model of volume overload-induced heart failure (6–8). Chronic volume overload (CVO) has been shown to induce rapid expression of atrial natriuretic peptide (ANP) mRNA and protein in cardiac tissue, as well as natriuretic peptide receptors (NPRs) (3, 22, 31, 35). Furthermore, ANP has been shown to cause degranulation in noncardiac mast cells (10, 29, 39). However, it is not known whether ANP activates cardiac mast cells. Accordingly, we performed in vitro and in vivo studies to investigate whether ANP directly stimulates cardiac mast cell degranulation. Isolated cardiac mast cells demonstrated no direct responsiveness to ANP. In the whole heart, infusion of ANP failed to cause mast cell degranulation, MMP activation, or changes in interstitial collagen levels. Similarly, administration of an NPR blocker, ananiti, to rats with an AV fistula did not prevent adverse CVO-induced remodeling.

MATERIALS AND METHODS

Animal Welfare

Adult male Sprague-Dawley rats were housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed to the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by our institution’s Animal Care and Use Committee. Anesthesia for the experimental procedure was induced via pentobarbital sodium (50 mg/kg ip).

Experimental Design

Isolated heart assessment of ANP effects. The experimental groups included an untreated control group of isolated hearts perfused with a saline bolus (n = 6) and a group of hearts perfused with ANP (225 pg/ml, n = 7; Sigma, St. Louis, MO). Experiments were performed using an isolated, blood-perfused heart preparation, as previously described (25). Briefly, the ascending thoracic aorta in the anesthetized rat was cannulated for continuous retrograde perfusion of the heart via an apparatus consisting of a pressurized perfusion reservoir (100–105 mmHg) and a collection reservoir connected in circuit to a support rat. The extirpated heart was attached to the apparatus for perfusion with oxygenated blood obtained from the support rat via a carotid artery catheter. The coronary venous effluent was collected in a reservoir and returned to the support rat via a jugular vein catheter. After removal of the left atrium, a highly compliant latex balloon was inserted into the ventricular chamber to obtain left ventricular (LV) pressure-volume (P-V) relationships. The proximal end of the balloon was connected via a short piece of tubing to a three-way stopcock that was used to adjust the balloon volume through one port during LV pressure measurement with a pressure transducer (Transpac IV, Abbott Critical Care Systems, North Chicago, IL) that was attached to the other port. Once the heart developed stable isovolumetric contractions, the balloon volume that produced an LV end-diastolic pressure (LVEDP) of 0 mmHg was used to determine baseline ventricular volume (V0). The volume in the balloon was then increased in 5- to 10-μl increments until an LVEDP of 25 mmHg was attained. The end-diastolic and peak isovolumetric pressures were recorded after each increase in balloon volume, with three to four data sets collected to ensure that the preparation was stable.

For assessment of the effect of ANP on coronary flow and mast cell degranulation, 1 ml of blood containing ANP was administered to the isolated heart of normal rats via the pressurized perfusion reservoir, which allowed the solution to mix with the reservoir blood to achieve a final concentration of 225 pg/ml ANP. This pathophysiological dose was selected on the basis of 1) preliminary observations of ANP levels in the infrarenal AV fistula model of CVO (19, 22), 2) baseline plasma ANP levels of 94.5 ± 12 pg/ml reported in previously published studies using rats (5), and 3) an expected two- to threefold increase in

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ANP characteristic of heart failure regardless of etiology (i.e., myocardial infarction or pressure or volume overload; for review see Ref. 1). Coronary venous effluent was collected for 3 min immediately before and after ANP administration to determine coronary flow. The coronary venous blood containing ANP was not returned to the support rat. P-V relationships were obtained for each heart before and after ANP infusion. We reported previously that compound 48/80 or endothelin-1 caused significant mast cell degranulation effects within this time period (14, 25). Control hearts underwent the same blood-perfused isolation procedure, with saline, instead of ANP, introduced into the perfusion reservoir. After completion of the functional studies, the atria and great vessels were removed, and the LV (including the septum) and right ventricle were separated. A complete transmural section of the LV at the midventricular level was placed in buffered formalin, and the remaining tissue was minced into 1-mm cubes and snap-frozen in liquid nitrogen for storage at −80°C. Wet-to-dry weight ratio differences expressed as percent myocardial water were determined using 100 mg of the frozen LV tissue.

Infrarenal abdominal aorta-inferior vena cava (AV) fistula. A ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava ~1.5 cm below the renal arteries. Both vessels were occluded proximal and distal to the intended puncture site, and an 18-gauge needle was inserted into the exposed abdominal aorta and advanced through the medial wall into the vena cava (7, 8). The needle was withdrawn, and the puncture site was sealed with surgical glue. Creation of a successful AV fistula became visually evident by pulsatile flow of oxygenated blood into the vena cava on release of the occlusion. The abdominal musculature and skin incisions were closed by 3/0 catgut and autoclips, respectively. The AV fistula model produces a biventricular blood overload with normal systolic conditions, no retrograde blood flow as in aortic or mitral valve insufficiency, and normal or slightly reduced aortic blood pressure. Throughout the remodeling process, there was no histological evidence of myocardial necrosis in this model (7). The NPR antagonist antinat (American Peptide, Sunnyvale, CA) was administered (10 µg·kg−1·day−1) via tail vein injection as previously described (15) 1 day before surgery and continued for the 3-day experimental protocol. Three days after surgery was selected as the end point, because we previously showed not only an increase in mast cell density but also, a significant increase in myocardial MMP-2 activity and a marked reduction in collagen volume fraction at this time (7).

Assessment of ventricular volume and function. In vivo ventricular volume and function were assessed using a high-fidelity Millar conductance catheter inserted into the LV via the carotid artery. After steady-state function data were collected, the vena cava was compressed, and the effects of the decrease in venous return and subsequent expansion of central blood volume were recorded. A small hypertonic saline bolus (0.2 ml, 15%) was introduced via a jugular catheter to provide a correction for parallel conductance. At the end of the protocol, a volume calibration curve was generated using a Millar calibration cuvette filled with heparinized blood.

Histology: mast cells and collagen. The transmural section of LV taken from the midventricle was processed for routine histopathology, whereby sequential 5-µm paraffin-embedded sections were stained with pinacyanol erthrosinate for the visualization of mast cell morphology (25) and with picrosiris red for quantification of myocardial interstitial collagen. LV interstitial collagen volume fraction (CVF), reported as the percentage of collagen relative to total myocardial area (7, 34), was determined in a blinded fashion using the ImagePro Plus Analysis System (Media Cybernetics, Silver Spring, MD) and established methods (37). Perivascular collagen was excluded from the analysis of CVF.

MMP activity. MMP activity in cardiac tissue extracts was analyzed using gelatin zymography performed by standard procedures using an SDS-PAGE matrix containing gelatin (1 mg/ml) (6, 14). Activity of the bands was quantified by densitometry (QuantityOne, Bio-Rad). All the zymograms had two lytic bands corresponding to standards for the proenzyme (68 kDa) and activated (62 kDa) forms of gelatinase A (MMP-2; Chemicon, Temecula, CA). The values obtained for MMP activity for each sample were normalized for their protein concentration measured using a Bio-Rad protein assay. Each gel was run in duplicate, and, for comparison of results from different gels, extract from the same control heart was used as a standard on all gels. The activity of the lytic bands in the other lanes of a gel was expressed as a percentage of this standard’s activity. Once normalized in this fashion, the percent activities from hearts belonging to each group (i.e., control and fistula) were averaged.

Isolation of cardiac mast cells. Anesthetized rats were secured on a board inclined at an angle of ~30° (head raised) from the surgical table. A tracheotomy was performed, and the rat was ventilated for the duration of the procedure. A ventral midline incision was made in the abdomen and extended to the level of the xiphoid cartilage. After completion of bilateral thoracic incisions, a lateral-to-medial incision of the diaphragm was made along the ventral aspect of the thoracic wall, leaving the falciform ligament intact. The left ventral thoracic wall was retracted medially to expose the heart, which was still encapsulated by the pericardium. A 24-gauge Teflon Baxter intravascular over-the-needle Quick-Cath catheter (1.6 cm) was then inserted into the pericardium, and the pericardial space was filled with room temperature Hanks’ buffer [Hanks’ balanced salt solution (HBSS): Hanks’ calcium- and magnesium-free salt solution, 13 mM HEPES, 607 U/ml of deoxyribonuclease I (Sigma), and an antibiotic-antimycotic mixture of penicillin G sodium, 10,000 mg/ml of streptomycin sulfate, and 25 mg/ml of amphotericin B (GIBCO BRL, Life Technologies, Grand Island, NY), pH 7.4]. Through the entry hole, the HBSS was removed, placed on ice into 15-ml Falcon tubes, and brought to even volumes with HBSS. The tubes were then centrifuged for 10 min at 1,000 rpm (4°C).

After centrifugation, the supernatant was collected and frozen at −80°C for later testing of spontaneous histamine release, while the pellet was reconstituted in 1 ml of Hanks’ complete balanced salt solution (HBSSMC). To determine the number of mast cells obtained from the isolation process, an aliquot from each sample was stained with toluidine blue for 20 min before all mast cells were counted on a hemocytometer. The concentration of mast cells was then calculated from the 1-ml volumes.

Peritoneal mast cell isolation. According to established methods (16), 20 µl of HBSS were injected into the peritoneal cavity, and the abdomen was gently massaged. The peritoneal fluid was aspirated and then diluted with HBSS and filtered through a 70-µm-pore nylon mesh cell strainer. After centrifugation (4°C), the cell pellet was resuspended in a known volume of HBSS. Aliquots of each sample were stained with toluidine blue for determination of mast cell number.

ANP treatment of mast cells. For determination of mast cell functionality, the isolated samples were analyzed for histamine concentration. Peritoneal and cardiac mast cell isolates were diluted with additional HBSSMC and then divided into four aliquots of 500 µl each in microcentrifuge tubes for treatment with ANP (Sigma). To allow for a constant concentration of mast cells across all treatment tubes, the isolates were diluted with additional HBSSMC, so when the sample was divided into 500-µl aliquots, it yielded ~4,000 mast cells per tube. The tubes were centrifuged again at 1,000 rpm for 10 min, and 200 µl of the supernatant were removed for measurement of spontaneous histamine release before treatment. The pellets were resuspended in the remaining 300 µl of buffer. After addition of 200 µl of HBSSMC containing 10−8, 10−7, and 10−6 M ANP to each sample, the samples were incubated in a shaking water bath at 37°C for 20 min at 60 rpm and then placed on ice for 10 min. Samples were then centrifuged (4°C, 5 min, 660 g), and the posttreatment supernatants were removed for measurement of histamine release in response to ANP. The pellets were resuspended in 500 µl of HBSSMC and sonicated twice at 10 s per sonication to release all the contents from the cells and, thereby, obtain a measure of unreleased histamine.
concentrations of ANP. In peritoneal mast cells, ANP induced an increase in histamine, as evidenced by a significant 32% increase in histamine release with $10^{-6}$ M ANP compared with control (Fig. 1). These results were consistent with previous reported findings (39). In contrast, no significant differences in histamine levels relative to control were observed in cardiac mast cells treated with the same doses of ANP. A known mast cell stimulant was used to ensure responsiveness of mast cells after the isolation process. Compound 48/80 significantly increased histamine release by 278% and 298% relative to control levels in cardiac and peritoneal mast cells, respectively.

### Isolated Heart Assessment of ANP Effects

LV cross sections from the isolated heart experiments were stained with pinacyanol ethrosinate and examined microscopically ($\times 400$) to determine the percentage of mast cell degranulation (Table 1). Degranulation was defined as the presence of disseminated granules external to the cell membrane and/or loss of membrane integrity vs. total myocardial mast cell number. The number of degranulated mast cells was not significantly altered in the ANP-treated hearts compared with control hearts (24.4 ± 5.6% vs. 25.8 ± 6.5%). Consistent with the limited appearance of cardiac mast cell degranulation, there were no significant changes in percent myocardial water in the ANP-treated hearts relative to control hearts (77.2 ± 2.0% and 74.1 ± 3.8%, respectively). Average coronary flow as measured by timed collection of coronary effluent immediately before and after ANP administration was not significantly altered (4.7 and 4.9 ml/min at baseline and after infusion, respectively). We next evaluated LV tissue from saline- and ANP-treated isolated hearts for histamine content. Analysis of saline- and ANP-treated hearts revealed no differences in the overall histamine levels (61 ± 10 and 82 ± 18 ng/ml, respectively, $P = 0.37$).

MMP-2 activity was also assessed in saline-treated (control) and ANP-treated isolated hearts. Densitometric analysis of zymography gels revealed a nonsignificant 14.7% increase in MMP-2 activity after ANP infusion relative to control hearts (Table 1, Fig. 2). Although MMP-9 bands were present in all groups, no significant difference in MMP-9 activity was observed between control and ANP-treated hearts. CVF was determined by confocal analysis of picrosiirius red-stained LV sections and reported as the percentage of collagen relative to total myocardial area. Determination of CVF in ANP-treated hearts revealed no differences relative to control (1.6 ± 0.2% and 1.4 ± 0.2%, respectively; Fig. 3).

Average LVEDP-LV end-diastolic volume (LVEDV) relationships before and after ANP administration are reported in Table 1. In ANP-treated hearts, LVEDV at 0 mmHg LVEDP

### Table 1. Body weight, LV weight, EDV, and function for isolated heart study

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>LV Wt, mg</th>
<th>$V_{0}$, μl</th>
<th>$\Delta V_{25}$, μl</th>
<th>$+\frac{dP}{dt}$, mmHg/s</th>
<th>$-\frac{dP}{dt}$, mmHg/s</th>
<th>MC Degranulation, %</th>
<th>MMP-2 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline</strong></td>
<td>273±24</td>
<td>627±52</td>
<td>242±20 (pre)</td>
<td>38±13 (pre)</td>
<td>4,513±1,591 (pre)</td>
<td>−2,599±489 (pre)</td>
<td>26±6</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td><strong>ANP</strong></td>
<td>273±30</td>
<td>675±33</td>
<td>256±15 (pre)</td>
<td>36±31 (pre)</td>
<td>3,790±1,120 (post)</td>
<td>−2,080±820 (post)</td>
<td>24±6</td>
<td>1.7±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. LV, left ventricle; EDV, end-diastolic volume; $V_{0}$, volume at which end-diastolic pressure (EDP) = 0; $\Delta V_{25}$, volume required to increase LVEDP from 0 to 25 mmHg; $+\frac{dP}{dt}$ and $-\frac{dP}{dt}$, maximum and minimum change in pressure over time; ANP, atrial natriuretic peptide (225 pg/ml); pre and post, before and after treatment. Matrix metalloprotease 2 (MMP-2) data reflect activity normalized to a single control across all gels.
(V₀) was not significantly different from baseline (248 ± 19 and 256 ± 15 µl, respectively). Furthermore, LV chamber compliance, as assessed by the volume required to increase LVEDP from 0 to 25 mmHg (ΔV₂₅), was not different after ANP administration (36 ± 3 vs. 33 ± 2 µl). Next, we evaluated the effects of ANP on functional parameters in the isolated hearts by comparing the minimum change in pressure over time (−dP/dt) and maximum change in pressure over time (+dP/dt) at baseline and 30 min after ANP treatment. Assessment of −dP/dt and +dP/dt revealed a negligible change in these values after ANP infusion (Table 1). Furthermore, evaluation of the slopes of the peak isovolumic pressure-LVEDV relationship, an accurate indicator of LV contractility (36), established that contractility of the ANP-treated hearts was not significantly different from baseline (1.8 ± 0.2 and 1.6 ± 0.2 mmHg/µl, respectively).

**Effects of ANP Receptor Blockade on Cardiac Remodeling in the AV Fistula Model of Volume Overload**

After 3 days of CVO, LV mast cell density in the untreated fistula group was not significantly different from that in the NPR blocker (NPRB)-treated fistula group (3.5 ± 0.6 and 2.7 ± 1.3 LV mast cells/mm² LV, respectively; Table 2). However, mast cell density in the untreated fistula and NPRB-treated fistula groups were elevated (1.75- and 1.35-fold, respectively) compared with sham-operated controls. Consistent with the increase in mast cell density, MMP-2 activity as assessed by zymographic analysis of LV tissue demonstrated a 2.7- and 2.6-fold increase in MMP-2 activity in both fistula groups relative to sham-operated hearts (Table 2, Fig. 2). MMP-9 bands were present in the three groups; however, no significant differences in MMP-9 activity were observed between sham-operated, untreated fistula, and NPRB-treated groups. CVF was also measured in these hearts to determine whether NPRB would result in attenuation of extracellular matrix degradation. Relative to sham-operated animals (1.7 ± 0.08%), both fistula groups demonstrated a distinct reduction in the amount of fibrillar collagen (1.4 ± 0.07% and 1.5 ± 0.1% for untreated fistula and NPRB-treated fistula groups, respectively, P ≤ 0.05 and 0.06 vs. sham; Fig. 3). No significant differences in CVF were observed between the NPRB-treated fistula group and the untreated fistula group.

Comparison of LV weights in sham-operated, untreated fistula, and NPRB-treated groups demonstrated only a modest amount of ventricular hypertrophy at this acute stage. However, as seen in Table 2, marked chamber dilatation was evident in both fistula groups. Relative to sham-operated hearts, NPRB resulted in LV chamber dilatation similar to the untreated fistula group reflected by a 95% and 72% increase in LVEDV, respectively (Table 2). This was mirrored by changes in cardiac output, which increased by 60% and 44% in NPRB-treated and untreated fistula preparations, respectively. Assessment of −dP/dt and +dP/dt (Table 2) demonstrated a modest increase in contractility in both fistula groups. Twenty-four-hour urine outputs were measured for all surgical groups to assess efficacy of ANP blockade by the NPR antagonist. Urine output was significantly diminished in the NPRB-treated fistula group compared with the sham-operated or untreated fistula group (8.4 ± 1.7 vs. 12.5 ± 4 and 13.3 ± 3 ml/day, respectively, P ≤ 0.05).

The observation that ANP receptor antagonism did not have an effect in vivo could possibly be due to the failure of mast cells to express the NPR. To address this issue, isolated cardiac mast cell samples were incubated with a fluorescent (TAMRA)-labeled synthetic ANP. Labeled ANP did not bind to mast cells identified by toluidine blue staining (Fig. 4), indicating that cardiac mast cells do not express receptors for ANP.

**DISCUSSION**

Recent investigations have shown a rapid and marked increase in ANP expression in heart failure patients and animal models of heart failure, regardless of etiology (i.e., myocardial infarction, pressure overload, or volume overload). It was concluded that this elevation in the myocardial natriuretic system was an adaptational, autocrine/paracrine response responsible for regulating cardiac remodeling (1, 17, 19, 22, 26). ANP, which has been shown to cause degranulation in non-cardiac mast cells (10, 29, 39), was originally identified as a member of a family of three separate peptide neurohormones,
ANP and brain natriuretic peptide (which are synthesized in the cardiomyocyte) and C-type natriuretic peptide (which is predominantly expressed in the brain), all of which act through cGMP-dependent receptors. Several studies using the AV fistula model of CVO have demonstrated rapid induction of ANP mRNA expression and protein in the heart, as well as NPRs (3, 19, 31). The goal of this study was to determine whether ANP is capable of directly stimulating cardiac mast cell degranulation, which in turn mediates the initial phase of cardiac remodeling, including MMP activation leading to degradation of the collagen network producing ventricular dilatation.

Effect of ANP on Cardiac Mast Cells

Yoshida et al. (39) and Opgenorth et al. (29) demonstrated, on the basis of histamine release, that mast cells were sensitive to ANP. However, they utilized peritoneal mast cells, and their primary focus was the ability of extracellular calcium to inhibit histamine release by maintaining membrane integrity in a manner similar to that seen with substance P. Chai et al. (10) demonstrated that increases in cytosolic cGMP induced in a dose-dependent fashion by ANP in peritoneal cells mediate intracellular calcium release and subsequent exocytosis of granules. They also concluded that extracellular calcium, which also increased intracellular calcium, may inhibit histamine release via the inhibitory action of cAMP or cGMP phosphodiesterase. Accordingly, we sought to determine the responsiveness of cardiac mast cells to direct stimulation by ANP. Consistent with findings of previous studies in which peritoneal mast cells responded in a concentration-dependent manner (10, 29, 39), 10^{-8} M ANP did not significantly increase histamine release compared with control (Fig. 1), whereas 10^{-6} M ANP induced a significant increase in histamine release from peritoneal mast cells. In contrast, isolated cardiac mast cells did not respond to ANP at either concentration. Although the isolation process of cardiac mast cells differs from that of peritoneal mast cells and, therefore, could affect the overall functionality of the mast cells, cardiac and peritoneal mast cells were equally responsive to the secretagogue compound 48/80. Using the isolated heart, we also evaluated the effect of ANP on cardiac mast cell activation.

Effects of ANP on the Isolated Heart

To complement the in vitro studies, we infused ANP into blood-perfused isolated normal rat hearts to evaluate its effects on cardiac mast cells. Previous findings from our laboratory demonstrated that a 30-min perfusion of the isolated heart with endothelin-1 or compound 48/80 induced 1) cardiac mast cell degranulation, 2) significant activation of myocardial MMPs, 3) myocardial collagen degradation, and 4) a moderate rightward shift in the P-V relationship (i.e., an indication of LV dilatation) (14, 25). With use of a similar approach, our present results indicate that ANP infusion (225 pg/ml) did not increase mast cell degranulation or MMP activation, nor did it shift the LV P-V relationship. This reinforces the in vitro findings that, in contrast to published findings on mast cells derived from other tissues, ANP does not directly cause cardiac mast cell activation in the isolated intact heart. However, these results do not rule out the possibility of longer-term in vivo ANP effects. We therefore utilized an ANP receptor antagonist to determine

Table 2. Body weight, LV weight, EDV, and function for surgical groups

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>LV Wt, mg</th>
<th>EDV, μl</th>
<th>+dP/dt, mmHg/s</th>
<th>−dP/dt, mmHg/s</th>
<th>MC Density, MCs/mm²</th>
<th>MMP-2 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>285±7</td>
<td>666±38</td>
<td>192±102</td>
<td>6,773±1,438</td>
<td>−6,656±2,032</td>
<td>2.0±0.9</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Fistula</td>
<td>289±10</td>
<td>686±51</td>
<td>375±62*</td>
<td>9,237±635*</td>
<td>−8,900±832</td>
<td>3.5±0.6*</td>
<td>2.7±0.1*</td>
</tr>
<tr>
<td>NRPB</td>
<td>278±12</td>
<td>714±54</td>
<td>453±168*</td>
<td>9,331±2,426</td>
<td>−8,509±1,781</td>
<td>2.7±1.3</td>
<td>2.6±0.1*</td>
</tr>
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</table>

Values are means ± SD. MC, mast cell; NRPB, natriuretic peptide receptor blockade. MMP-2 data reflect activity normalized to a single control across all gels. *P < 0.05 vs. sham.

Fig. 4. Fluorescent-labeled ANP immunostaining for natriuretic peptide receptor type A. A: toluidine blue staining specific for mast cell granules. B: cell surface natriuretic receptors of non-mast cells binding fluorescent-labeled ANP. C: merged image of toluidine blue- and fluorescent-labeled cells. Magnification ×40. Arrows indicate mast cells.
whether it could prevent mast cell-mediated effects secondary to an AV fistula.

Effects of ANP Receptor Antagonism on Cardiac Remodeling in the AV Fistula Model of Volume Overload

Previously, we were able to demonstrate not only a temporal association between mast cell number and MMP activation but, also, a cause-and-effect relationship between mast cell secretory products and MMP activity in the AV fistula model of CVO (6–8). Once released into the myocardium after mast cell activation/degranulation, mast cell preformed products (e.g., tryptase and chymase) were able to cleave and activate latent MMPs, which subsequently caused collagen matrix degradation. Furthermore, the use of mast cell membrane-stabilizing compounds, such as cromolyn and nedocromil, abrogated these effects, thereby minimizing MMP activity and subsequent loss of collagen (9, 14). Huang et al. (19) demonstrated that, after creation of an AV fistula, circulating levels of ANP increased significantly within the first 24 h and remained elevated after 5 wk of CVO. This marked elevation of tissue ANP occurred within the first 24 h and was associated with subsequent LV hypertrophy. The use of the ANP-deficient Nppa−/− mouse revealed that ANP was a key regulator of the myocardial hypertrophic response to volume overload (23). Consistent with those findings, others used the cardiac-specific NPR-A-deficient mouse or inhibition of neutral endopeptidases responsible for the degradation of ANP to demonstrate that endogenous ANP exerts critical autocrine/paracrine effects on ventricular hypertrophy and fibrosis (30, 33). Additionally, several investigators have suggested that ANP has a direct cardioprotective anti hypertrophic role in the failing heart (28, 38). The use of ANP receptor antagonists, cGMP analogs, and NPR-A knock-out mice demonstrated that endogenous ANP inhibits cardiomyocyte hypertrophy and that inhibiting ANP worsens ventricular remodeling (18, 20, 21, 23, 27). Consistent with those findings, our results demonstrate that, for 3 days after creation of an AV fistula, administration of anantin, an NPR antagonist, led to exaggerated LV hypertrophy relative to the untreated fistula; however, neither cardiac mast cell density nor MMP activation was affected.

The findings outlined above and the fact that cardiac mast cells did not bind the fluorescent-labeled ANP peptide indicate that it is plausible to conclude that cardiac mast cells do not respond to ANP.

In conclusion, although ANP appears to play a critical regulatory role in the heart’s hypertrophic response secondary to a physiological insult, our findings indicate that ANP does not cause cardiac mast cell activation. Furthermore, these results also indicate that ANP is not responsible for the acute induction of cardiac mast cell-mediated ventricular remodeling in our rodent model of heart failure. Even though mast cells have a common origin, i.e., from bone marrow precursors, their phenotype is apparently determined by the microenvironment. This difference in endogenous tissue level signaling and reciprocal expression, in all likelihood, accounts for the differences in responsiveness to ANP between peritoneal and cardiac mast cells, even though both are classified as connective tissue-type mast cells. To our knowledge, this is the first study to show differences in the responsiveness of peritoneal vs. cardiac mast cells. However, future studies are warranted to assess the role of ANP in chronic ventricular remodeling and decompensation, as well as the contribution of other circulating factors (e.g., endothelin-1, TNF-α, and transforming growth factor-β) that are associated with heart failure.

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